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## EFFECT OF A MODIFIED WOW-SYSTEM AND EMBRYO DENSITY DURING IN VITRO CULTURE ON DEVELOPMENTAL RATE OF CAPRINE EMBRYOS

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The most common and efficient systems used in IVP of embryos in small ruminants consist in large number of oocytes (20-70) matured, fertilized and cultured together in the same medium, thus preventing traceability of the origin of the oocytes. Individual oocyte in vitro fertilization and embryo culture has been proven to be unsuccessful. Therefore, our objective was to trace the donor animal by handling small groups of oocytes (n=5) instead of the oocyte individually.

Oocyte-cumulus complexes collected from slaughterhouse derived goat ovaries were matured, fertilized and zygotes were cultured in SOF-BSA in presence of serum to the blastocyst stage as previously described (Cognie *et al.*, 1995, AETE, 146). To improve the developmental rates, we tried to use a modified Well-of-the-Well system (WOW) consisting in culturing groups of 5 presumptive zygotes (PZ) in microwells within a conventional four well dish. We compared the following 4 experimental culture conditions: 5 PZ in a 15µL drop (5/15d; n=118), 5 PZ in a single WOW in a 15µL drop (5/15wow; n=93), 15 PZ in a 50µL drop (15/50d; n=160); 15 PZ in 3 WOW (3x5 PZ) under the same 50µL drop (15/50wow; n=339). These groups allowed us to separately assess the effect of the culture system (CS ; Drop vs WOW) and the effect of the number of PZ per group (NPZ ; 5 vs 15) on the developmental rate, while the standard in vitro culture (IVC) system consisting in 25 PZ in a 25µL drop (25/25d; n=423) was also performed as a control group. The effects of the CS and the NPZ, as well as their interaction on cleavage and blastocyst rates were analyzed by generalized linear models for categorical variables. Comparisons of groups vs. the standard IVC system were performed by the Dunnett's test for proportions. Both, CS and NPZ had a very significant effect (p<0.002 and p<0.0001, respectively) on the developmental rate in favor of the WOW system and the group of 15, with no significant interaction. Cleavage rate did not differ between all groups. Developmental rate at day 8 was higher in the 25/25d control and 15/50wow groups (58% and 59% respectively). The 15/50d group yielded an intermediate rate of development of 49%, and the 5/15d and 5/15wow groups showed significantly lower developmental rates than the control group (22% and 41%, P < 0.001 and P < 0.05, respectively).

These results demonstrate the beneficial effects of culturing large number of PZ together and the suitability of a modified-WOW system, which allows small groups of PZ to share the same medium, to achieve traceability. The promising results of the modified WOW-system make it interesting to use for in-vivo derived oocytes from OPU to handle oocytes by donor and assure their filiations.

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